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Isolation of *Leptospira* in the urine of occupationally predisposed human groups in Sokoto state, Nigeria

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Target Audience*: Health policy makers, community medicine officers, para-veterinarians, animal scientists, laboratory technologists, researchers, biomedical students and the general public*

A**bstract**

This research undertook a prospective bacteriological survey for Leptospira in humans. The objective was to investigate the possibility of Leptospira circulating in apparently healthy but known occupationally predisposed human groups as a subset of the general population. Standard and globally applicable culture isolation methods were used to screen random occupationally vulnerable groups recruited in the study. By informed consent and participation, urine samples were subjected to Ellinghausen McCollough Johnson Harris (EMJH) culture in a ration of 2:18 sample against medium as previously used with high isolation rate by researchers. A total of 176 human urine samples were analyzed, demographical factors such as age, sex, seasons and occupation were carefully recorded for each sample. An overall prevalence of 10.2% was obtained. The abattoir workers recorded a prevalence of 5.7% as did the dog handlers with a prevalence of 29.7%. Veterinarians, previously reported as constituting the prime risk group recorded a prevalence of 2.7% in contrast to animal handlers which serve as nurses with a prevalence of 10.0%. There was significant association of rainy season with infection (P<0.05). The evidence shows various occupationally predisposed human groups presents asymptomatic infection hence serving as possible carriers of Leptospira. Although serovars were not determined, detection of Leptospira underscores ***pr****ospective surveys as the first step in determining infection status in population so that asymptomatic carriers will be isolated then treated.*

K***eywords****: Leptospira, Occupation, Diagnosis, Zoonosis, Outbreak, Prevention*

Description of Problem

Infections of domestic and wild animals that are transmitted directly or by indirect means to humans are a major cause of morbidity and mortality worldwide (1), including those in Nigeria, a nation with the largest human population, economy and a substantial landmass in Africa (2). Leptospirosis is a globally important zoonosis and zoo-anthroponosis caused by spirochetes of the genus *Leptospira* (3). Human infection results from exposure to infected urine of carrier mammals, either directly or by contaminated soil or water, thus animal shedders pose significant public health risk (4).

Diagnosis is often missed in resource limited settings due to low index of suspicion amongst clinicians, the broad spectrum of clinical presentations often mimicking many other infectious agents and associated with barely existing diagnostic facilities (4). Although globally important and dreaded, leptospirosis remains under-diagnosed and under-reported in Africa, and consequentially, is overlooked as public health priority (5). It is not considered as a priority reportable disease in most countries in Africa including Nigeria.

In Africa, zoonotic infections are both directly responsible for human illness and death and indirectly impact human well being as a result of reduced livestock productivity (6). In tropical developing

countries, where most people are poor and live in crowded conditions with poor sanitation and awareness, human transmission through exposures to urine of *Leptospira*-infected livestock, companion animals (dogs), peridomestic rodents and or wild animals are common (7).

In Africa, approximately 154 human cases of leptospirosis occur annually with an incidence of six human cases reported annually in Nigeria (8); this, no doubt, represents a gross underestimation due to paucity of evidenced-based data.

In diagnostic laboratories, real-time PCR is increasingly-been used compared to conventional PCR. The technique is faster, requires less laboratory personnel and is performed in a closed system, thereby minimizing DNA cross-contamination (9). Although despite the several real-time PCR assays that have been described for the diagnosis of leptospirosis, only very few have been validated for use in canine urine samples (10). In his research (11) reported two real-time qPCR assays targeting the 16S ribosomal RNA and the subsurface lipoprotein (*LipL*32) gene for prompt diagnosis of leptospirosis using urine samples.

Human leptospirosis is a reportable disease in many developed countries (12). The disease is a well-recognized occupational hazard to farmers, dairy workers, abattoir workers (12) and several other predisposed groups like veterinarians, animal handlers, animal scientists, and butchers reported in many other studies. Leptospirosis is reported to be prevalent in developing countries, despite that, human leptospirosis is hardly considered as differential even when symptoms of the disease are present nor is a random survey for *Leptospira* commonly done in reported occupationally predisposed human subjects in Nigeria.

The aim of this research was to survey targeted occupationally predisposed human groups for the presence of pathogenic *Leptospira.* This is the first prospective screening for *Leptospira* in humans in the studied region in Nigeria.

Materials and Methods

Sample Size and Sample Collection

The study was carried out in Sokoto State, northwestern Nigeria (12-13.9667, 4.1333-6.9) (13) as part of a wider study on the survey for *Leptospira* serovars in dogs with a questionnaire for dog owners. The sample frame was generated to constitute all Local Government Areas (LGAs) that constitute Sokoto metropolis which consists of Sokoto South, Sokoto North, Wammako, Kware and Dange Shuni. An overall sample size (N=342) was calculated for the State which is made up of 23 Local Government Areas (LGAs), This sample size was halved to obtain sample size "n" for the State metropolis (made up of 5 LGAs) where clinics and abattoirs are located. The total sample size (n=176) was divided among these LGAs and occupationally predisposed groups: veterinarians, animal handlers, dog handlers, clinicians, and abattoir workers. These groups were sampled randomly by sampling and skipping days alternately. Samples were requested based on consent and participation in the study. A total of 10ml of urine was requested using 25ml Eppendorp tubes, each collected urine sample was tested on-site with a litmus paper, where acidic, another 10ml of bicarbonate buffer was added, and then the sample batch was labelled and transported to the laboratory.

Culture Isolation and Microscopy

Each urine sample was cultured according to methods described by (14) at the Central Diagnostic Laboratory, National

Veterinary Research Laboratory, (NVRI), Vom-Plateau State, Nigeria. The Ellinghausen-McCullough-Johnson Harris (EMJH) (Difco) basal media was prepared by dissolving 2.3g of powdered media per liter, 10% glycerol, pH 7.4. Fetal calf serum was added to commercial *Leptospira* enrichment (Difco) prepared according to methods described by (14) and (15). Final liquid EMJH was prepared by dissolving 100ml enrichment and 10ml fetal calf serum per liter of EMJH basal media. The media was sterilized through a 0.22um filtration system. A total of 2mL of urine samples were inoculated into 18mL EMJH liquid media and incubated at room temperature. Cultures were sub-cultured every 10 days and viewed weekly by dark field microscopy using the Olympus BX 51 at magnifications x40, 60 and 100. Data were analyzed using logistic regression in STATA 2011. Odds ratio was used to determine the likelihood of infection as it associates with various demographical variables.

Result

Out of 176 human urine samples collected, 18(10.2%) were positive for *Leptospira* after culturing and dark-field microscopy. Relative prevalence varied across the different occupationally predisposed human groups. The dog handlers represented the highest isolation rate with 11(29.7%) positive. Clinicians (which is a term for technologists processing samples) recorded 3(5.3%) positive while abattoir workers had 2(5.7%) positive. The veterinarians and animal handlers are the closest to dogs in the clinics, 1(2.7%) and 1(10.0%) were positive for veterinarians and animal handlers respectively.

Males had higher isolation rate with 17(9.9%) positive in contrast to 1(25.0%) female positive. The different age groups presented varying isolation rates and susceptibilities to infection. The young adults (aged 1to 20years) had 2(18.2%) positive compared to the middle aged adults (aged 21-40) that recorded 13(9.6%) positive. The advanced ages (greater than 40 years) recorded 3(10.3%) positive for the organism.

The three seasons prevalent in the study area showed varying prevalence to *Leptospira.* The rainy season (May to October) had 14(14.1%) positive that was statistically significant ($P<0.05$) compared to the harmattan season (November to February) which recorded a prevalence of 2(4.8%). The hot season (March to September) recorded isolation rate of 2(5.7%).

Discussion

Bacterial zoonosis represents a class of neglected human infections responsible for a significant proportion of febrile illness or sometimes asymptomatic colonization (1). Research has projected that present and future generations may face the highest risk of exposure to zoonotic infections in human history, requiring the global community to address the impact it may have on life expectancy and quality of health (2). In Nigeria, a countrywide survey for leptospirosis cases in humans reported a prevalence of 20.4% (16). This is comparatively higher compared to the 10.2% prevalence recorded in this present study for plausible reasons that the study was localized to a region of Nigeria and with different disease determinants. Another variant factor is the difference in times the studies were conducted: spanning about two decades, and the difference in the techniques adopted for the two referred studies. The present research undertook a culture isolation technique based on a globally adopted media choice and ration that is previously used and highly accurate.

Table 1: Bacteriological prevalence of *Leptospira* in different human occupational high-risk groups

Variables	Culture +ive (%)	Culture –ive(%)	OR (95% CI)	P
Occupation				
Vet	1(2.70)	36(97.30)	0.29(0.01, 11.88)	0.46
CL	3(5.30)	54(94.70)	0.93(0.11, 24.13)	0.89
DH	11(29.70)	26(70.30)	4.13(0.58,100.5)	0.19
AW	2(5.70)	33(94.30)	0.61(0.04, 19.59)	0.71
AH	1(10.00)	9(90.00)	1.00	
Gender				
Male	17(9.90)	155(90.10)	0.37(0.10, 1.81)	0.19
Female	1(25.00	3(75.00)	1 .00	
Age				
1-20	2(18.20)	9(81.80)	1.00	
21-40	13 (9.60)	123(90.40)	0.47(0.10, 3.47)	0.39
>40	3(10.30)	26(89.70)	0.53(0.07, 5.05)	0.54
Season				
RS	14(14.10)	85(85.90)	2.95(0.72, 20.01)	0.04*
HA	2(4.80)	40(95.20)	0.90(0.09, 9.03)	0.92
Hot Season	2(5.70)	33(94.30)	1.00	

Keys
* Statistically significant at P<0.05
CL: Clinicians; DH Dog handlers; AW Abattoir workers; AH Animal Handlers; RS Rainy Season; HA Harmattan

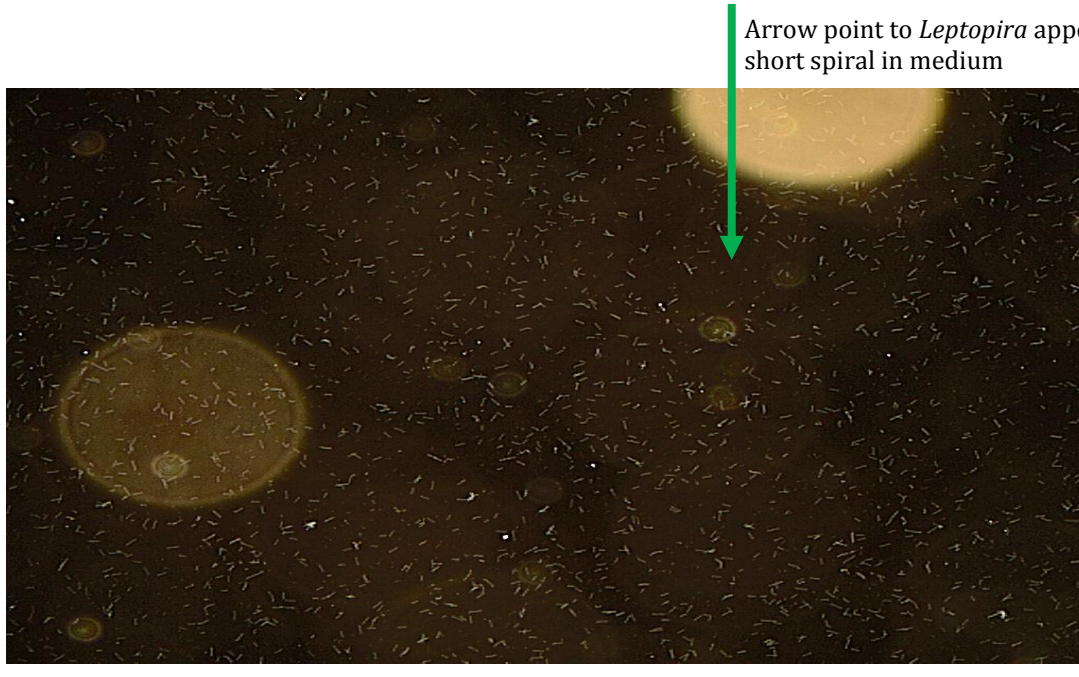

Fig 1. Photomicrograph of *Leptospira* in Liquid EMJH from Urine Samples of Occupational Predisposed Human Subjects on Dark field Microscope

Leptospirosis is particularly common in the tropical areas where people and animals live in close contact, and where warm conditions favour environmental survival and transmission of the pathogen (17). In Nigeria, two regional background sero-surveys in healthy people yielded leptospiral prevalence ranging from 13.5% in eastern State (18) to 18.0% in the Central State of Jos (19, 20). These are reported prevalence in humans and data as old as two decades is hardly reflective of the current situation and justifies the need for updated surveys targeting pathogen culture as serological data is unreliable in some instances.

The persistence of leptospires in a patient's urine for more than one year indicates the urine may be a sample in chronic infection or carrier animals, as reported in other text. Research undertaken in the 80s reported the isolation of pathogenic *Leptospira* in blood-negative samples on animal and human hosts. The fact that this isolation was observed even after treatment with antibiotics such as penicillin, amoxicillin, and vibramycin according to the authors, poses the question of whether the usual treatment of leptospirosis with these antibiotics can remove all bacteria from the human kidneys. The persistence of leptospirosis in the kidneys after treatment with antibiotics has been described for cattle for three decades (21) and hamsters (22) and may also occur in humans (23).

A comprehensive metadata review of leptospirosis in Africa (20) identified 97 studies describing acute human leptospirosis (n=46) and animal leptospirosis (n=51) in just 26 of 51 African countries, exposing the paucity of research and diminished disease reporting in Africa. Numerous studies in that account cited acute human leptospirosis with non-specific fever and jaundice associated with patients leading to the recovery of pathogenic *Leptospira* in urine samples collected. This raises the probability and specter of humans shedding leptospires.

In our study of 176 human urine samples, an overall prevalence of 10.2% (Table 1) was obtained in comparison with 18% reported in Jos, Plateau State, Nigeria by (12). The difference in timelines may explain the variation as human settlements, social systems as well as microbial ecology may have had changes over time. Another plausible reason for variation may be the technique used. (12) reported the isolation of serovars hardjo for the first time in Nigeria, putatively because of the correlation of serovar hardjo as bovine adapted serovars and for dairy workers' close contact with cows in Jos (Nigeria), where a lot of them were sampled. The study reported a prevalence of 29.5% in abattoir workers in contrast to our study that determined a prevalence of 5.7% in abattoir workers. Dog handlers which is a euphemism for butchers in ruminants recorded a prevalence of 29.7% (Table 1) plausibly because of their daily contact with sick dogs which are often the ones brought for slaughter in remote areas within the study area.

Our study showed that middle age (21-40years) and ages above 40 years with a recorded prevalence of 9.4% and 10.3%, respectively (Table 1), maybe the most susceptible, however, that was statistically insignificant ($P>0.05$) when compared to younger age group. Plausible reasons may be that the referred age groups were the most active in outdoor hours compared to others. A study from Vietnam showed that in rural endemic areas, exposure begins at a young age, with a substantial rate of sero-conversions remaining largely asymptomatic (24). Transmission in rural areas is related to increased rainfall, livestock holding, and farming. In this study, the rainy season, with

a prevalence of 14.1% (Table 1), recorded a statistically significant (P<0.05) correlation with infection in occupationally predisposed hosts. This is consistent with the reports of (25), who recorded a time series of increased risks of leptospiral infection with the rainy season compared to other seasons over a 15 years period in Canada. The seasonal variation can help to guide screening, surveillance and other health preventive policies to minimize infection in humans and animals.

Studies in the past in Nigeria, very few albeit, reported significantly higher prevalence among coal mine workers (18), abattoir workers (19), and farmers (16). A recent risk analysis among abattoir workers revealed low levels of awareness, and high levels of risk-of-exposure-prone practices (26).

In Ghana, Leptospirosis has been appreciably studied in humans (27). A published report (28) demonstrated a sero-prevalence of 33.3% in 460 healthy inhabitants from the Ashanti district, with a significantly higher prevalence in bush farmers and cocoa plantation workers. The authors also demonstrated leptospirosis in 3.2% of 190 patients with jaundice and/or undifferentiated fever in the same region. In 1973, a 21.2% sero-prevalence was reported in ill people from the Ashanti and Volta regions (29). It is therefore, pertinent for developing countries to have a policy of continuous strategic survey for evidence of leptospiral dissemination in various locations.

Isolation of *Leptospira* from randomly sampled clinical specimens from occupationally predisposed human groups has important public health implications as it points to a subset of individuals in the general population shedding the pathogen for the susceptible human or animal host to contract.

Prospective surveys underscore the need for population data that will be useful for designing prevention and intervention programs. Such data can serve as a warning system of a potential rise in scale and frequency of disease. The data obtained in this study hopefully will sensitize health authorities and policymakers on the presence of pathogenic *Leptospira* circulating in a population that may appear apparently healthy.

Conclusion and Applications

1. *Leptospira* is circulating in apparently healthy human occupational groups in Sokoto Stste Nigeria with an overall prevalence of 10.2% in a prospective survey
2. The different occupational groups are exposed in varying degrees to the infection with the dog handlers having the highest prevalence of 11(29.7%), so is season with the rainy season presenting highest prevalence of 14(14.1%) which was statistically significant (P<0.05).
3. The Middle age group (21-40years) presented the highest risk of infection with reported prevalence of 13(9.6%).

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